

Policy & Procedure (P&P)

Policy Title :

Direct Antiglobulin Test "Direct Coombs"

Department	Index No.	Scope
Laboratory & Blood Bank	LAB-071	All Blood Bank and Lab Staff
Issue Date	Revision NO	Effective Date
1432/6/4	3	1440/06/20
Review Due Date	Related Standard NO.	Page Number#
1442/06/20	CBAHI (LB.66)	7

01. Policy:

01.1. Direct antiglobulin (Coombs) test is used for detection of all or autoantibodies present on the RBCs.

02. Definition:

N/A

03. Purpos :

03.1. Demonstration in vivo coating of red cells with gamma globulins, in particular IgG and C3dg.

04. Procedure:

Anti-human globulin serum will react with red cells sensitized with gamma globulin (red blood cells antibody) or component of human complement and cause agglutination of the red blood cells. The direct antiglobulin test is used to detect in vivo red blood cell sensitization.

Washed red blood cells from the patient are directly tested with the antiglobulin serum. The direct antiglobulin test is useful for:

1. Diagnosis of hemolytic disease of the newborn.
2. Diagnosis of autoimmune hemolytic anemia.
3. Drugs induced hemolysis.
4. Investigation of transfusion reactions.

Note: Some drugs cause positive direct antiglobulin test.

Direct antiglobulin (Coombs) test can be done by any one of the following techniques:

A) Tube Method

1. Technique

1.1. Specimen Requirements

Blood drawn into EDTA is preferred (to prevent the fixation of complement in vivo) but oxalated, citrated or clotted whole blood may be used. The blood sample should be tested as soon as possible after collection and should not be stored. Testing must be completed within 24 hours. No patient preparation is required.

1.2. Materials

- 1.2.1. Glass test tubes and slides
- 1.2.2. Automatic cell washer
- 1.2.3. Disposable Blood Bank pipettes
- 1.2.4. Isotonic normal saline 0.85 NaCl
- 1.2.5. Marker pencil or pen
- 1.2.6. Microscope

1.3. Reagents

- 1.3.1. Broad spectrum – Anti-human serum for antiglobulin test comb's control cells.
- 1.3.2. IgG sensitized cells (CCC).

1.4. Method

- 1.4.1. Prepare a 2-5% suspension of red blood cells in isotonic saline.
- 1.4.2. With a Disposable Plastic Pipettes add one drop of the cell suspension to a labeled tube.
- 1.4.3. Wash the cells with normal saline 3 to 4 times.
- 1.4.4. Add two drops of anti-human serum and mix.
- 1.4.5. Centrifuge for 15 sec at 3000 rpm.
- 1.4.6. Re-suspend the red blood cells by gentle agitation, examine macroscopically for agglutination or hemolysis then examine microscopically.
- 1.4.7. If DAT is positive, test the positive cells with Anti-IgG and Anti-C3d, AHG reagents to characterize the types of proteins coating the red cells.
- 1.4.8. Add Coombs Cell Control to all negative antiglobulin tests by adding one drop of coombs control cells (CCC). Re-centrifuge and observe for macroscopic agglutination. These cells must agglutinate for a negative test

result to be valid.

1.4.9. Grade the result and record it in the log book.

1.5. Reporting Results

1.5.1. Positive reaction due to IgG sensitization should be positive immediately after anti-human serum is added. They will sometimes become weaker after room temperature incubation so all tests must be read immediately.

1.5.2. A negative test result may be reported only when coombs control cells agglutinate when added to the negative test.

1.6. Limitation

1.6.1. Causes of false negative test

1.6.1.1. Failure to wash cells adequately to remove all plasma proteins which will neutralize the antihuman globulin serum.

1.6.1.2. Contamination of Coombs reagent by extraneous protein. Do not use finger or hand to cover tube during mixing.

1.6.1.3. High concentration of IgG Para proteins in test plasma, protein may remain even after multiple washes.

1.6.1.4. Bound IgG may dissociate from red cells and leave little IgG to be detected.

1.6.1.5. Agglutination of IgG-coated cells is weakening by time, centrifuge and read immediately.

1.6.1.6. Improper reagent storage.

1.6.1.6.1. AHG may lose reactivity if frozen or if stored at temperatures above 6° C.

1.6.1.6.2. AHG may become bacteriological contaminate

1.6.1.7. Improper procedures

1.6.1.7.1. Under centrifugation.

1.6.1.7.2. Failure to add AHG reagent

1.6.1.8. Too heavy red cell concentration may mask weak agglutination.

1.6.1.9. Improper serum / cells ratios.

1.6.1.10. Saline

1.6.1.10.1. Low pH of saline can decrease sensitivity (optimal pH 7.0-7.2).

1.6.1.10.2. Some antibodies may require specific saline temperature. Use at both 37° C and 4° C.

1.6.2. Causes of false positive test

1.6.2.1. Cells agglutinated prior to washing

1.6.2.2. Particles or contaminants

1.6.2.2.1. Dust or dirt in glassware may cause clumping.

1.6.2.3. Improper procedure

1.6.2.3.1. Over centrifugation

1.6.2.3.2. Complement components

may bind to cells from
clot of CPDA-1 during
storage at 4° C. use fresh
EDTA samples.

1.6.2.3.3. Complement may attach to cells in specimens collected from infusion lines.

1.6.2.3.4. Septicemia in a patient or bacterial contamination of specimen may cause a false positive.

1.6.2.3.5. Red cells from clots refrigerated in contact with serum may give these false positives. This can be avoided by using red cells from anticoagulated specimens such as EDTA and by completing testing as soon as possible after collection.

Note:

A positive DAT does not necessarily mean that a person's red cells have shortened survival. Small amounts of both IgG and complement appear to be present on all red cells. That's why interpretation of positive DATs should include patient's history, clinical data, and the results of other laboratory tests.

Elevated levels of IgG or complement have been noted on the red cells of patients with sickle cell disease, β -Thalassemia, renal disease, multiple myeloma, autoimmune disorders (including SLE) AIDS, and other diseases with elevated serum globulin or blood urea nitrogen (BUN) levels with no clear correlation between positive DAT and anemia

1.7. Quality Control

Reagents are to be tested with appropriate positive and negative controls daily and the results recorded. Refer to "Daily Quality Control" procedure.

B) Gel Micro typing System

1. Principle

A specific red cell solution is added to the gel contained in a special micro tube. The gel acts as a trap, the free red blood cells pellet in the bottom of the tube while agglutinates are trapped(fixed)in the top of the gel for hours.

2. Equipment/materials

- 2.1. ID Centrifuge.
- 2.2. ID working table
- 2.3. ID pipettor EP-3/FP-2/FP-3
- 2.4. ID Dispenser
- 2.5. ID Suspension tubes
- 2.6. ID Disposable tips
- 2.7. Test tubes

3. Reagents

ID Diluent 2 (modified LISS)

4. Micro typing Cards

LISS/Coombs (Profile: -6 IgG/C3d Polyspecific Coombs tests)

5. Sample

EDTA Red cell concentrate/Whole blood

6. Test Procedures

- 6.1. Allow all reagents to reach room temperature before use.
- 6.2. Identify the ID-micro typing card with the patient name and number and remove the aluminum foil.
- 6.3. Prepare a 0.8% suspension of patients red cells as follow:
 - 6.3.1. 10-micron red cell concentrate + 1 ml ID- diluents 2 or
 - 6.3.2. 20-micron whole blood + 1 ml ID- diluents 2 mix well. It is best prepared cell suspension from cell concentrates (packed cells) to maintain greater consistency. The use of whole blood to prepare cell suspensions will be more

variable as PCV differs between blood samples.

- 6.4. Add 50 U of red cell suspension to the appropriate micro tube.
- 6.5. Centrifuge the micro typing card for 10 minutes in the ID-centrifuge.
- 6.6. Interpret the result.

7. Interpretation

- 7.1. Positive reaction, grade 1-4, in the micro tube indicates positive DAT.
- 7.2. Negative reaction in the microtube indicate negative DAT.

8. Quality Control

Reagents and microtubes are to be tested with appropriate positive and negative controls daily (ID-Internal Quality Control) and the results recorded. See "Daily Quality Control" for procedure.

C) DCT using TANGO Optima System (Fully Automated)

1. Principle of the test

Solid screen™ II Strip plates are composed of test strips (12 strips with 8 wells each) that have been coated with Protein A.

Protein A has a high binding affinity for the Fc region of immunoglobulins.

The method used for the Direct Chooms test on the Solid screen™ II Strip is solid phase adherence. If red blood cell alloantibodies and/or autoantibodies are present in the sample to be tested they will bind to the patients red blood cells.

Unbound antibody is removed by washing.

After adding Anti-Human- Globulin specially formulated for the Solid screen™ II assay and centrifugation Anti-Human-Globulin forms a link between the protein A on the surface of the microwell and the patients red blood cells Thereby a layer of red blood cells will form on the surface of the microwell.

But if no red blood cell alloantibodies or autoantibodies are present in the sample, the cells will not get coated with antibodies and therefore will not form a cell layer on the surface of the microwell but sediment to the bottom of the well to form a cell button.

2. Reagents

- 2.1. Solid screen™ II Strip Plates with 12 strips per plate. The strips are coated with protein A.
- 2.2. MLB 2 Modified LISS 2 (Low Ionic Strength Solution) is an antibody enhancement media specifically formulated for use with the Solid screen™ II test system, The MLB 2 is used in the preparation of a 1% cell suspension of sample red blood cells.
- 2.3. AL severs Solution Used as a suspension medium for red blood cells.

- 2.4. Anti-Human Globulin (Anti-IgG Solid screen™ II) The Anti-Human Globulin is used to demonstrate the presence of antibodies bound to the donors red blood cell that do not cause direct agglutination.

3. Materials

- 3.1. Glass test tubes
- 3.2. (N) rack of the tango Optima machine
- 3.3. Calibrated serologic centrifuge

4. Sample

EDTA Anticoagulated whole blood.

5. Test Procedures

- 5.1. Centrifuge Patients blood sample & put them in (N) Rack.
- 5.2. Open the sample door & put the(N) Rack in its correct place.
- 5.3. Push the Rack button to select the desired rack on the screen.
- 5.4. Press manual entry button & write the ID number of patient's sample.
- 5.5. Press accepted entry button.
- 5.6. Press (DCT) button to select the test & close the sample door.
- 5.7. Press start button to start the test.
- 5.8. After the end of the test validate the results.
- 5.9. Print out the results.

6. Quality Control

Control Set (QC) contain test erythrocytes with defined antigen pattern for ABO, Rh (D), phenotyping {Rh (CEce) and Kell} as well as isoagglutinin for reverse typing.

05. Responsibilities :

All Blood Bank Staff of Al-Qunfudah General Hospital

06. Equipment & Forms

Coombs Test Form

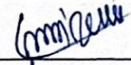
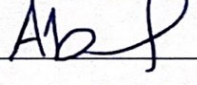
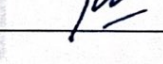
07. Attachment :

N/A

08. Reference

- 08.1. Tango Optima manual book.
08.2. Manual for BB in Arab countries.

Preparation , Reviewing & Approval Box

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